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Commentary

Gammaherpesviruses and "Hit-and-Run" Oncogenesis

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Two gammaherpesviruses are known to infect humans. Both are predominantly latent. Both were first discovered in tumor specimens and both carry one or more transforming genes. They are the Epstein-Barr virus (EBV) and the Kaposi's sarcoma herpesvirus (KSHV), also known as human herpesvirus 8. First discovered in association with endemic Burkitt's lymphoma, EBV is the etiological agent of infectious mononucleosis and has a long-recognized association with nasopharyngeal carcinoma and post-transplant lymphoproliferative disease.[1–4] More recent but well established associations include a subset of Hodgkin's disease,[5–7] nasal lymphoma,[8] smooth muscle tumors in immunocompromised patients,[9,10] a subset of gastric carcinoma,[11,12] AIDS primary central nervous system lymphoma,[13] a subset of other AIDS lymphomas, and a subset of peripheral T cell lymphomas.[2,14] Less well established associations have recently been reported in hepatocellular and breast carcinomas.[15–17] KSHV was first discovered in Kaposi's sarcoma in AIDS patients but has also proven to be associated with all other epidemiological forms of Kaposi's sarcoma.[18] The virus is also associated with primary effusion lymphoma and a subset of Castleman's disease.[19–21] An association with multiple myeloma has been reported but remains a subject of contention.[22]

Whereas human papillomaviruses and human T-cell leukemia virus type 1 (HTLV1) are found integrated into cellular DNA in tumors, the gammaherpesviruses generally persist as episomes in tumor cells. The episomes, although tethered to cellular chromatin by viral encoded nuclear proteins, are not continuous with cellular DNA. Present evidence suggests that EBV episomes are lost when there is no selective pressure for their maintenance. Episomal maintenance has been studied in tissue culture with recombinant plasmids and in tumor-derived cell lines. In tissue culture, human cells expressing the Epstein-Barr nuclear antigen-1 (EBNA1) protein will replicate recombinant bacterial plasmids containing an EBV origin of latency replication once with each cell cycle.[23,24] If the plasmid carries an antibiotic resistance gene, the plasmid will be stably maintained in the presence of antibiotic selection. However, in the absence of antibiotic selection, a percentage of cells will lose the plasmid with each generation, and ultimately the cultured cells will be plasmid-free. Similarly, it has been shown in a Burkitt's lymphoma-derived cell line (Akata) that after cloning in soft agar, some clones will lack the viral episome.[25] These clones grow more slowly, are less resistant to apoptosis, and are not tumorigenic in nude mice, in contrast to the parent cell line. In bulk culture, Akata cells that have lost episomes are presumably simply overgrown by cells that retain their episomes and thus have a growth advantage. Loss of episomes in some other Burkitt's cell lines has not been associated with loss of a malignant phenotype.[26] One possibility is that in some tumors, chromosomal translocations and point mutations have supplanted EBV as a driving force for proliferation or resistance to apoptosis, whereas in others EBV remains essential to the malignant phenotype. In this regard, it might be noted that the typical chromosomal breakpoints associated with endemic and non-endemic Burkitt's lymphoma differ somewhat at a molecular level.[27] Although Burkitt's cell lines typically retain their episomes in tissue culture, other tumors may consistently lose their episomes in culture. Thus, undifferentiated nasopharyngeal carcinoma is an epithelial cell tumor consistently associated with EBV. In tissue culture, nasopharyngeal carcinoma cell lines typically lack viral episomes. We have recently investigated this phenomenon in the NPC-C666 cell line and have shown that episomal loss corresponds to activation of lytic cycle viral expression with consequent shutoff of transcription of the EBNA1 protein required for episomal maintenance.[28] Kaposi's sarcoma cell lines in tissue culture also lose viral episomes, although the molecular biology underlying this loss has not yet been studied.

It has recently been shown that in some cases of episome loss from Burkitt's lines in tissue culture, fragments of the EBV genome are incorporated into cellular DNA.[26] Furthermore, in a series of non-endemic Burkitt's

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lymphomas, similar fragments of the EBV genome were found in tumors that by standard criteria would be classed as virus-negative by EBER *in situ* hybridization or EBNA1 immunohistochemistry insofar as these parts of the genome were not retained.[29] Thus, EBV may play a role in endemic and sporadic Burkitt's lymphoma, as recently proposed by John Sixbey at the Stohlman Scholar Symposium of the Leukemia Society of America (November 12–13, 1999, New York City). He argued that a rare subclone of proliferating cells that has spontaneously lost EBV at some point after tumor initiation but is now capable of sustaining virus-independent growth may have a survival advantage through avoidance of immune surveillance. Thus, in geographic regions where Burkitt's lymphoma occurs only sporadically (in populations with good nutrition and presumed vigorous immune surveillance), the tumor is a rare malignancy and is for the most part EBV-negative, whereas in other areas, among populations with malnutrition, chronic parasitic infection, and impaired immune surveillance, Burkitt's lymphoma is much more common and is predominantly EBV-positive.

The possible role of EBV in Hodgkin's disease has tantalized epidemiologists, virologists, and clinicians for several decades. The variation of the bimodal age incidence curve of Hodgkin's disease with the level of economic development suggests that the incidence of the disease in children and young adults is related to age of infection with a common virus.[30] Case control studies suggested that factors in the childhood environment that influenced timing of exposure to infection also influenced the risk of Hodgkin's disease.[31] Small family size and low housing density, which might be associated with delayed exposure to infection by a ubiquitous infectious agent, predicted an increased risk of Hodgkin's disease. The development of Hodgkin's disease after late or delayed exposure to a virus was specifically likened to the paralytic consequences of poliovirus infection, which followed the infection more commonly when it occurred in later adolescence or adulthood rather than in infancy or childhood. The idea that EBV infection *per se* rather than another ubiquitous agent might be linked specifically to Hodgkin's disease was supported by several sorts of observations. Seroepidemiological studies that showed that antibody titers to EBV antigens were elevated in Hodgkin's disease patients both at the time of diagnosis and several years in anticipation of diagnosis.[32] A history of infectious mononucleosis was associated with about a threefold increase of risk for young adult Hodgkin's disease.[33,34] In case reports, Hodgkin's disease developed in close association with primary EBV infection.[35]

The whole picture appeared to be internally consistent when monoclonal EBV genomes were found in the Reed-Sternberg cells of Hodgkin's disease in a subset of patients.[7,36–39] However, the story has not been quite so neat as might have been hoped. The distribution of EBV-positive Hodgkin's disease cases is almost an inverse image of that predicted by the epidemiological data. Among young adult cases, where the poliovirus hypothesis is most relevant, EBV positivity in tumor tissue is generally lowest.[6,40] The association of Hodgkin's disease with EBV is highest in underdeveloped countries, children, and the aged. The whole picture might be unified by an extension of Sixbey's hypothesis as generated to explain the Burkitt's lymphoma data. EBV might be an important cofactor for Hodgkin's disease in general, but the viral episome might be lost from malignant cells, where tumor progression had rendered the viral episome unnecessary for survival, and in patients with relatively intact immune systems, where the presence of the viral episome and expression of associated viral antigens rendered the tumor susceptible to immune surveillance.

This was presumably the thinking that led Staratschek-Jox and colleagues to apply an *in situ* hybridization technique to apparently EBV-negative tumors to Hodgkin's disease in search of fragments of the viral genome.[41] The methods applied are state of the art, and the answer appears to be clear. In contrast to the report of viral DNA fragments in sporadic Burkitt's lymphoma, no fragments of the viral genome were detected in the cases of Hodgkin's they studied. If the virus is involved in the pathogenesis of these EBV-negative cases, it seems to have made a clean escape, not leaving behind any DNA evidence for the lab to investigate further. Studies of Burkitt's, nasopharyngeal carcinoma, and Kaposi's sarcoma cell lines suggest that such clean escapes are possible; thus, a "hit-and-run" role for EBV or KSHV in many diseases cannot be excluded. The challenge now is to devise investigative strategies that might lead to the conclusive identification of hit-and-run perpetrators or to exclude them definitively. The difficulty in devising such strategies has for the most part stopped investigators in the field from discussing the possibility of gammaherpesvirus hit-and-run oncogenesis in print, but the idea has led to many late-night discussions at scientific meetings. Developing strategies to prove or exclude hit-and-run oncogenesis associated with episomal loss remains an interesting challenge.

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